

## THE "TORCHES" (CONGENITAL DISEASES) PROGRAMME

### 1. IN WOMEN OF CHILD-BEARING AGE

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#### SYNOPSIS

The term "TORCHES" describes the congenital diseases *Toxoplasmosis*, *Rubella*, *Cytomegalovirus* (CMV) infection, *Herpes simplex* and *Syphilis*. 365 women of child-bearing age of whom 181 were nurses and 184 were members of the general population were examined, as part of a wider collaborative programme concerned with the importance of "TORCHES" infections in Malaysia as a cause of congenital infection and foetal damage.

Antibodies against toxoplasma were found in 23% of them, against rubella in 64%, CMV in 83%, herpes simplex in 79% and against syphilis in 5%. The frequency of antibodies was similar in the nurses and the general population, except for the toxoplasma and syphilis antibodies which were commoner in the general population. Toxoplasma infections were also found to be more prevalent in Malays than in Chinese and Indians in Malaysia, while rubella infection was least common among Malays in the general population. Possible reasons for these differences are discussed.

Of all the infections suspected of being able to cause congenital malformations, toxoplasmosis, rubella and cytomegalovirus (CMV) infection appear to be most important (White and Sever, 1967). Herpes simplex and syphilis may also cause congenital defects but much less frequently. Suspicion has also been attached to chicken-pox, measles, mumps and hepatitis, but a cohort study for congenital malformations following these infections (Siegel, 1973) failed to show any significant causal relationships.

The term "TORCHES" employed in the title of this paper has been coined to represent the following congenital diseases: *Toxoplasmosis*, *Rubella*, *CMV* infection, *Herpes simplex* and *Syphilis*. The "TORCHES" programme is an antibody survey carried out collaboratively by Winnie Cheah (toxoplasmosis) Dora S.K. Tan (rubella), H. Stern (CMV and herpes simplex) and K.D. Sukumaran (syphilis) in three parts, namely surveys of:

- (i) women of child-bearing age, 14 to 44 years old,
- (ii) mentally retarded children, 0 to 5 years old, comparing them with normal children of the same ages, and
- (iii) pregnant women throughout their course of pregnancy and the babies born to those women who undergo primary infection.

This paper reports the results obtained in the first part of the study, namely, the prevalence of "TORCHES" infections among women of child-bearing age in Peninsular Malaysia.

#### MATERIALS

Two groups of women were surveyed, (a) nursing staff at the General and Maternity Hospitals in Kuala Lumpur, and (b) women in the general population from various parts of Peninsular Malaysia. The first group was sampled with the intention of including any of them who become pregnant in part (iii) of our investigation.

Altogether, 365 women were examined, 181 nursing staff and 184 general population. Blood samples were obtained and each was separated and distributed for serological testing to the various laboratories. However, not all the sera could be tested as some were lysed or contaminated. Moreover, some sera were anti-complementary in the complement-fixation tests for CMV and herpes simplex and their results could not be presented (see Table).

#### METHODS

##### Toxoplasmosis

The antigen, a strain of *Toxoplasma gondii*, was maintained in infected mice in the laboratory, and slides were freshly prepared and frozen until required for use, usually within a month.

The indirect fluorescent antibody technique (IFAT) was performed according to the technique described by Palmer (1970) and Remington (1969). However, Cooke's microtitre apparatus and anti-human immunoglobulin supplied by Burrough Wellcome were employed in our method.

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TABLE I  
DISTRIBUTION OF "TORCHES" ANTIBODIES IN WOMEN OF CHILD-BEARING AGE IN MALAYSIA

## (a) Nurses

Racial group	TO			R			C			HE			S		
	+	Tot.	%	+	Tot.	%	+	Tot.	%	+	Tot.	%	+	Tot.	%
Malay	16	65	25	47	65	72	52	63	83	47	63	75	0	21	0
Chinese	14	96	18	61	96	64	71	88	81	69	88	78	1	60	2
Indian	3	20	15	9	20	45	16	19	84	14	19	74	0	4	0
Total	33	181	18	117	181	64	139	170	82	130	170	76	1	85	1

## (b) General Population

Malay	31	79	39	43	80	54	31	36	86	28	36	78	8	52	15
Chinese	16	76	21	56	78	72	40	47	85	43	47	91	0	44	0
Indian	4	29	14	21	29	72	16	18	89	12	18	67	0	19	0
Total	51	184	28	120	187	64	87	101	86	83	101	82	8	115	7

## (c) Total

Malay	47	144	33	90	145	62	83	99	84	75	99	76	8	73	11
Chinese	30	172	17	117	174	67	111	135	82	112	135	83	1	104	1
Indian	7	49	14	30	49	61	32	37	86	26	37	70	0	23	0
Total	84	365	23	237	368	64	226	271	83	213	271	79	9	200	5

TO= toxoplasmosis; R= rubella; C= cytomegalovirus; HE= herpes simplex; S= syphilis

A reaction showing at least a Grade II peripheral fluorescence was considered positive, whereas in a negative reaction the organism was stained entirely red. Titres of 1:16 and above were regarded as significant for previous infection.

**Rubella**

Haemagglutination inhibiting antibodies were estimated with the Rubella Test Kit designed by Behringwerke AG. Sera were treated by manganous chloride-heparin for removal of non-specific inhibitors (Cooper *et al*, 1969).

**Cytomegalovirus and herpes simplex**

Complement-fixing antibodies were estimated by the standard microtitre technique, using 2 units of complement and overnight incubation at 4°C. The CMV antigen was prepared as a cell-associated antigen from the Ad 169 strain of virus (Stern and Elek, 1965). The herpes simplex antigen was grown in tissue culture and supplied by the Central Public Health Laboratories, Colindale, London. All sera were inactivated at 56°C for 30 minutes and examined at 1 in 8 dilution.

**Syphilis**

Two tests, the non-treponemal and the treponemal tests were performed as recommended by the W.H.O. and C.D.C. (Center for Disease Control, U.S.A.).

*(i) Non-treponemal test or VDRL (Venereal Disease Research Laboratory) test*

This is a qualitative slide flocculation test and detects a lipophilic antibody called "Reagin" which is contained in the IgG and IgM fractions of the immunoglobulins. As it is not immunologically specific, weak false positive results were frequently encountered. It was therefore necessary that "reactive" or "weakly reactive" sera be further subjected to a quantitative and confirmatory test, the treponemal test.

*(ii) Treponemal test or FTA-ABS (Fluorescent Treponemal Antibody Absorption test)*

This test is highly specific for *Treponema pallidum* and is sensitive for all stages of the disease. The method used is described in the "Manual of tests for syphilis, 1969" published by the U.S. Department of Health, Education and Welfare, 1969.

## RESULTS AND DISCUSSION

The overall frequency of antibodies in the two groups of women examined was 23% for toxoplasmosis, 64% for rubella, 83% for CMV infection, 79% for herpes simplex and 5% for syphilis (Table). No significant differences were found between the nurses (group a) and the women of the general population (group b) except in the case of toxoplasmosis and syphilis. Toxoplasmosis antibody was present in 18% of the nurses and in 28% of the general population, and syphilis antibody, present in 1% of the nurses and 7% of the general population.

The distribution of antibodies was similar for all three racial groups in the population, Malay, Chinese and Indians, except again for toxoplasmosis; the frequency of this antibody was almost twice as great in the Malay women as in the Chinese ( $P < 0.05$ ). The number of Indian women tested in both groups (a) and (b) was much smaller than the other racial groups and this might, at least partially, explain the finding of a generally lower frequency of antibodies in these women.

The infection rate for toxoplasma was highest in the Malay women, both nurses and general population, confirming the earlier studies carried out by Tan and Zaman (1973). They reported toxoplasma antibody in 25% of Malays, 20% of Indians, and 5% of Chinese. This could be ascribed to the larger number of domestic cats kept by the rural Malay population and their closer association with them. Although pigs are known to have the highest toxoplasma infection rate among domestic animals (Zaman *et al*, 1967) and the Chinese consume more pork than any other meat, they usually cook their meat very thoroughly and avoid infection by this route. Since nurses are not likely to be exposed unusually to toxoplasmosis in their hospitals, the distribution of antibodies among the three racial groups of nurses remains the same as in the general population.

The frequency of rubella antibody was not markedly different in the Malay and Chinese nurses, but in the general population, it was significantly lower in the Malays. The most probable explanation is that the Malays live mostly in rural areas which are much less congested, while the Chinese abound in the urban areas, which are more densely populated and offer greater opportunities for spread of infection. On the other hand, the Malay nurses would be exposed to much more infection in their hospital and clinics than in the rural areas from which they come and would soon equal their colleagues in the frequency of rubella antibody.

Our finding of rubella antibody in 64% of women of child bearing age in Malaysia agrees with the previous study of Lam in 1972 (60%). These figures are low when compared with those reports from most western countries. Thus, antibodies have been found in 80% or more of adult women in

Europe, the United Kingdom, the U.S.A., Australia and Canada, as well as in Singapore and some South American states (Rawls *et al*, 1967; Dowdle *et al*, 1970). However, much lower rates were found in the rural areas of Japan, Trinidad, Jamaica, Panama and rural Peru, as well as in Hawaii (Sever *et al*, 1965) and Thailand (Thongcharoen *et al*, 1970). The possible reasons suggested for the lower levels of infection; lesser density population and geographical isolation, are not applicable to Bangkok or Kuala Lumpur. Another possible explanation offered was the tropical climate common to most countries with low rubella antibody rates which might make respiratory spread of a labile virus more difficult.

Contrary to expectation, population with low frequency rubella antibody are no more susceptible to rubella outbreaks than those with immunity levels of 80% and above. Moreover, outbreaks in these countries have been associated with relatively low attack rates, and a correspondingly small increase in the incidence of congenital anomalies in the population (Dowdle *et al*, 1970). For example, although a minor outbreak of rubella occurred in Singapore, during the period November, 1969, to July, 1970, Malaysia, which is linked to Singapore by a  $\frac{3}{4}$ -mile causeway, was completely unaffected. The importance of rubella as a cause of congenital anomalies in Malaysia is not known but is currently being studied in part (ii) of the "TORCHES" programme.

In Japan, rubella immunity levels are low and extensive epidemics have been observed since the spring of 1965, but the incidence of congenital-rubella syndrome has been extremely low. Kono *et al* (1969) attributed this to infection by strains of rubella virus that are antigenically different from those occurring in the U.S. and are also less virulent for rabbits. Whether Malaysian strains of rubella virus belong to the same antigenic groups as those found in Japan is yet to be studied.

CMV and herpes simplex virus infections are both obviously widespread in Malaysia, the frequency of antibodies in the adult population being similar to that found for similar populations in other countries (Buddingh *et al*, 1953; Krech *et al*, 1971; and Juel-Jensen and MacCallum, 1972). The actual ages of acquisition of antibodies in the three racial groups in Malaysia is now being studied (Tan and Stern, unpublished). Despite the prevalence of infection, cytomegalic inclusion disease and the various clinical manifestation of herpes simplex have not been frequently described here, either because they escape recognition or are mostly subclinical. The further "TORCHES" programme is designed to determine whether there is any association between these infections and congenital anomalies or mental retardation, as been described in Europe and the U.S.A. (Stern *et al*, 1969; Hanshaw, 1971; and Stern and Tucker, 1973).

Out of 200 serum specimens examined for syphilis, 5% were found positive. However, this incidence must be interpreted with caution. Although there is a global increase in the incidence of venereal disease, yaws is still prevalent in Malaysia. The two organisms, *Treponema pallidum* of syphilis and *Treponema pertenue* of yaws are morphologically indistinguishable and both diseases induce the formation of indistinguishable anti-treponemal antibodies. Only a further in-depth epidemiological study can project for certain what proportions of the above positive reactors are due to yaws and what proportions are due to syphilis.

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